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Research Article

Isotopic niches reveal the impact of topmouth gudgeon and gibel carp on native crucian carp

Marek Šmejkal[1], Kiran Thomas[1], Zuzana Šmejkalová[1], Yevdokiia Stepanyshyna[1,2], Daniel Bartoň[1], Sandip Tapkir[1], Travis Meador[2,3], Mojmír Vašek[1]

1 *Institute of Hydrobiology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic*
2 *Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic*
3 *Institute of Soil Biology and Biogeochemistry, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic*
Corresponding author: Marek Šmejkal (marek.smejkal@hbu.cas.cz)

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Abstract

Invasive species pose a major threat to natural ecosystems and directly outcompete many native species, placing them at imminent threat of extinction. The topmouth gudgeon (*Pseudorasbora parva*) is on the EU's blacklist of invasive freshwater species and threatens biodiversity, especially in wetland and floodplain ecosystems, aquacultures and village ponds. The crucian carp (*Carassius carassius*) is native to Europe and its populations have declined in large part of its native range, with invasive gibel carp (*C. gibelio*) suspected as a major cause of its decline. Invasions by topmouth gudgeon have been implicated in the decline of crucian carp populations but this still needs to be verified. The aim of this study was to evaluate by the experimental approach the competitive interaction between the two species, topmouth gudgeon and crucian carp, focusing on isotopic niche sizes and their overlap in syntopy. A four-month mesocosm experiment was performed to determine the isotopic niche of crucian carp and topmouth gudgeon living alone and in syntopy. Additionally, stable isotope data were collected at the sites where the two species co-occur to compare niche sizes and overlaps. Experimental data showed that the isotopic space of topmouth gudgeon responded more flexibly (reducing niche size at syntopy) than that of the crucian carp and confirmed a high isotopic niche overlap between the species. Field studies have shown that topmouth gudgeon has invaded the isotopic niche of the crucian carp, especially when another invasive species, the gibel carp, lived in the community (25% at 40% ellipse area and 50% at 95% ellipse area). When only the topmouth gudgeon and crucian carp were present in the field, the overlap was lower (3% and 48%, respectively) and directional overlap modelling showed that the crucian carp was more likely to invade the isotopic niche of topmouth gudgeon than vice versa. The data indicated that competition between crucian carp and topmouth gudgeon is likely, especially in syntopy with other invasive species. This study shows that the feeding plasticity of topmouth gudgeon likely facilitates its establishment outside its native range and, due to high isotopic niche overlap, threatens native fish with similar feeding ecology with competitive displacement.

Key words: Biological invasions, freshwater conservation, interspecific competition, invasive species, SIA, stable isotope analysis

Introduction

Wetland and floodplain ecosystems are amongst the most threatened freshwater ecosystems (Davidson 2014; Colvin et al. 2019; Tickner et al. 2020). Wetlands have been disconnected from the river main channels by river floodplain

canalisation and land conversion, while the construction of dams has resulted in the loss of natural flow dynamics (Grizzetti et al. 2017; Hayes et al. 2018). Pollution and eutrophication of these ecosystems further degrade their functioning (Kingsford et al. 2016) and can act in synergy with other stressors, negatively impacting native species (Gorule et al. 2024). Furthermore, wetland ecosystems often harbour invasive species (Glińska-Lewczuk et al. 2016) and many freshwater fish species which have adapted to these conditions are at risk of severe decline (Sayer et al. 2011; Van Wichelen et al. 2022). Interaction with invasive species can cause various changes for native fish populations, such as a reduction in growth and recruitment (Tapkir et al. 2022), a change in isotopic niche position (Declerck et al. 2002; Busst and Britton 2017), increased predation rates (Reshetnikov 2003), enhanced disease transmission (Andreou and Gozlan 2016) or habitat displacement (Šmejkal et al. 2023a). All of these effects can lead to a reduction or extinction of the population of native species.

Fish from the Cyprinidae family were the first to be spread by humans outside their native ranges due to aquaculture, angling and ornamental fish breeding purposes and now pose a serious problem for biodiversity worldwide (Meller and Crowl 2006; Rylková et al. 2013 Souza et al. 2022). One of the most widespread invasive fish in Europe is the topmouth gudgeon *Pseudorasbora parva* (Gozlan et al. 2010; van der Veer and Nentwig 2015). It was accidently imported with aquaculture stocks in the early 1960s (Bănărescu 1964) and is now widespread across most river courses in Europe (Gozlan et al. 2010). The topmouth gudgeon reaches high abundances in the absence of piscivorous fish and reduces the amount and size of available zooplankton for other fish species (Musil et al. 2014; Kajgrová et al. 2022). As it often inhabits remnants of former floodplains and small bodies of water in Europe (Glińska-Lewczuk et al. 2016), it poses an immediate threat to the native fauna of already threatened ecosystems (Pollux and Korosi 2006; Carpentier et al. 2008). Topmouth gudgeon has several life history traits that make it extremely successful: maturation in the first year of life (Britton et al. 2008), batch spawning strategy with a very long reproductive period, parental care (Maekawa et al. 1996) or feeding on a variety of foods, including small benthic and zooplanktonic organisms as well as detritus (Wolfram-Wais et al. 1999; Britton et al. 2010; Kajgrová et al. 2022). It also copes well with pathogens and transmits diseases that are new to the native fauna (Gozlan et al. 2010; Spikmans et al. 2020). In terms of competition with other fish species, the topmouth gudgeon has been shown to invade the isotopic niches of omnivorous fish and its isotopic trophic range is very wide (Britton et al. 2010; Jackson and Britton 2013; Balzani et al. 2020), which poses a major threat to the already rare limnophilic fish of floodplain pools.

Stable isotope analysis has become a useful tool to assess the competitive relationship between invasive and native species (Post 2002; Haubrock et al. 2020). The isotopic niche of animal tissue integrates the assimilated dietary history of an organism over time (Post 2002; Jackson et al. 2011, 2012). Isotopic values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) allow the assessment of a species' diet and, thus, the size of its ecological food niche (Balzani et al. 2020 Haubrock et al. 2020).

There is a general trend in decline of native fish species of floodplain ecosystems in Europe (Aarts et al. 2004), partly due to habitat loss associated with anthropogenic control of floodplain flow dynamics (Arthington 2012). Amongst those declining fish is the formerly common fish species - crucian carp (*Carassius carassius*) (Sayer et al. 2011 Tarkan et al. 2016,; Fedorčák et al. 2023), which found

secondary suitable ecosystems in man-made ponds, flooded mining pits and stone quarries and was common in these systems until the end of 20th century (Sayer et al. 2011; Tarkan et al. 2016; Šmejkal et al. 2024). While their disappearance from floodplains can be attributed to the loss of primary habitat, the primary cause of this decline in secondary pond ecosystems is likely competition for resources with invasive cyprinids, such as the gibel carp (*C. gibelio*), goldfish (*C. auratus*) and common carp (*Cyprinus carpio*) (Copp et al. 2010; Busst and Britton 2017; Tapkir et al. 2023). However, the interaction of the crucian carp with the invasive topmouth gudgeon, which overlaps with their habitat requirements, has not yet been investigated in detail. Additionally, interaction of the crucian carp with topmouth gudgeon often occurs together with the presence of a second invasive species, the gibel carp (Lusková et al. 2010). Thus, field evidence encompassing interaction of these three species may provide additional insight into threats to the native crucian carp.

The extent of isotopic niche overlap between the invasive topmouth gudgeon and the declining crucian carp was investigated in the mesocosm experiment, with an aim to estimate the shift in isotopic niche position in syntopy compared to allopatry. In addition, two populations of crucian carp and topmouth gudgeon living in syntopy were sampled in the field together with four populations, where the gibel carp was present as a second invasive species.

Specifically, we hypothesised that:

i. Due to omnivorous food sources in both species and dominance of zooplankton and benthos in their diet (de Meo et al. 2022; Kajgrová et al. 2022), we expected that the isotopic niche of topmouth gudgeon overlaps to a large extent with the isotopic niche of the crucian carp.
ii. Due to a small maximum size and relatively small mouth of the topmouth gudgeon and its known reliance on zooplankton (Musil et al. 2014), we expected that, on average, it feeds more on pelagic prey, such as small zooplankton than the crucian carp.
iii. As the topmouth gudgeon is a very successful invasive species that is known for its broad and flexible dietary niche (Gozlan et al. 2010; Jackson and Britton 2013), including facultative parasitism on other fish species (Oberle et al. 2019), we expected that it has a larger and more flexible isotopic niche compared to the crucian carp.
iv. Finally, we expected that, due to increased competition over shared resources with the presence of a third fish species in the community, the gibel carp (Tapkir et al. 2023), the overlap of the isotopic niches of crucian carp and topmouth gudgeon will be larger.

Materials and methods

Mesocosm experiment

A mesocosm experiment was designed to test the size of the niches under conditions of syntopy and allopatry between the invasive topmouth gudgeon and the native crucian carp. The experiment was conducted using an array of 24 outdoor mesocosms at the Institute of Hydrobiology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czechia. To provide near-natural conditions, zooplankton and phytoplankton were collected from a nearby pond in České

Budějovice (48.9671469°N, 14.4528125°E) and transferred to two separate 500 litre circular vats kept outdoors. The phytoplankton and zooplankton inoculum were kept in the vats filled with water from the fish ponds for two days. Each mesocosm (rectangular shape, 98 cm length × 91 cm height × 90 cm width, made of high-density polyethylene) received 800 litres of aged tap water and 10 kg of sand. Two litres of the well-mixed phytoplankton and zooplankton inoculum were then added to each tank on 19 April 2023. The set-up was maintained for one month before the fish were added to stabilise the system for phytoplankton and zooplankton production.

The fish for the mesocosm experiment were collected on 7 May 2023 in the Smilovice and Ujezdec ponds (49.8669103°N, 14.9854556°E and 49.9006147°N, 14.9486450°E; topmouth gudgeon and crucian carp, respectively). The fish were transported to České Budějovice and kept in 300 l aquaria under laboratory conditions at 20 °C and a photoperiod of 12L:12D for a 12-day acclimatisation and antiparasitic treatment (Costapur, Sera, Heinsberg, Germany). They were fed 1% of body weight in dry food (C-3 Carpe F, Skretting, Stavanger, Norway) per day.

To determine the overlap of isotopic niches under single species and syntopy conditions, three treatments (crucian carp, topmouth gudgeon and syntopy) were distributed in a randomised block design with eight replicates per treatment. This arrangement was chosen to obtain a sufficient number of replicated mesocosms and a sufficient number of individual fish in each treatment. Length and weight of individual fish (0.1 g accuracy, SI-132-3 balance, Excell, New Taipei City, Taiwan) were recorded at the beginning and end of the experiment. The initial fish biomass in each tank was roughly 8 g, depending on fish size variability (mean ± standard deviation (hereafter SD), crucian carp = 7.42 ± 0.62 g; topmouth gudgeon = 8.22 ± 0.71 g, SL = 88 ± 12 mm; syntopy = 8.22 ± 0.39 g). Crucian carp and topmouth gudgeon used for the experiment had an SL = 35 ± 6 mm and a weight of 1.29 ± 0.73 g and a SL = 44 ± 3 mm and a weight of 1.36 ± 0.24, respectively. Six fish were used in each mesocosm (6 crucian carp, 6 topmouth gudgeon or 3 and 3 in syntopy treatment, i.e. a total of 72 fish per species). The initial biomass of 8 g corresponds to a population density of 80 $kg.ha^{-1}$, allowing for growth up to the carrying capacity of the mesocosm. The experiment ran for four months, from 19 May to 19 September 2023 and the mesocosms were monitored for transparency at the beginning, middle and end of the experiment using a Sneller tube (Tapkir et al. 2022). The treatments achieved a transparency of 43 ± 5, 45 ± 9 and 44 ± 9 cm for crucian carp, topmouth gudgeon and syntopy treatments, respectively. In addition, four data loggers (HOBO Pendant Temperature/Light 64 K Data Logger, Onset Computer Corporation, Bourne, Massachusetts, USA) were placed in four randomly chosen mesocosms in the middle of the water column to continuously monitor water temperature during the experiment (mean = 20.5 ± 3.0 °C, min = 13.4 °C, max = 28.5 °C).

At the end of the experiment, a total of 80 fin clips were collected (20 topmouth gudgeon and 20 crucian carp living in the syntopy treatment and 20 individuals of each species living in the single species treatment, 2–3 fish per species and mesocosm unit). As young individuals with a high growth rate were used, the stable isotope turnover during the warmest four months of the growing season should ensure a reasonable estimate of the individual isotopic niche position (Vander Zanden et al. 2015; Franssen et al. 2017; Busst and Britton 2018). This approach has been chosen to avoid unnecessary mortality in very small fish used for the experiment.

Field data collection

Fish were collected at six sites in the Czechia (small lentic waters with surface area between 0.01 and 0.20 ha, Table 1, Fig. 1) using fish traps (25 × 25 × 45 cm; 4 mm mesh size with two small funnel-shaped entrances of 6 cm diameter at each end) and "umbrella traps" (95 cm diameter, 8 small funnel-shaped entrances) baited with a combination of dog food pellets and bread slices. The nets (two fish traps and two umbrella traps) were set before sunset (5–7 pm) and checked in the morning (9–11 am, approximately 15 hours of exposure) (Thomas et al. 2023). The fish caught were then measured (SL; mm) and weighed (W; g). Three to four scales of crucian carp and gudgeon were removed with the blunt edge of tweezers from the top of each fish, just below the leading edge of the dorsal fin and above the lateral line. If the invasive gibel carp (*Carassius gibelio*) were present at the site, its scales were also collected for subsequent stable isotope analyses. The scales were dried and stored in paper envelopes. A total of 78 crucian carp, 82 topmouth gudgeon and 56 gibel carp from six sites were analysed (Table 1).

Stable isotope analysis

Eighty fin clips from mesocosms from the experimental part of the study were subjected to stable isotope analysis (SIA). To compare the experimental results with the field data, scales from the field data were used, as scales are an alternative to dorsal muscle or fin clips for SIA and lead to comparable results (Vašek et al. 2016; McCloskey et al. 2018). This reduced the damage caused to the critically-endangered crucian carp during data collection in the field.

A total of 216 sampled scales were selected from the six different sites (Table 1, Fig. 1). Up to four scales from each individual fish were submerged in distilled water and gently cleaned from epidermal skin, mucus and adhered material with a scalpel under a binocular microscope. Subsequently, the scales and fin clips were oven-dried at 60 °C for 24 h. Due to the small size of some fish scales and fin clips and their limited number available for analysis, they were not homogenised into a powder, as this would lead to the loss of some of the valuable material. Instead, the samples were cut into fine slices (like a pie) and 0.363–1.084 mg of the sample was then weighed into a tin capsule (Tapkir et al. 2023). Since a comparative analysis of acidified freshwater fish scales did not show a substantial effect of the inorganic C fraction on the resulting $\delta^{13}C$ values of total C versus organic C in fish scales (Ventura and Jeppesen 2010), we decided not to decalcify our scale samples by acid treatment. The stable isotopic ratio of C and N in the scale samples were determined at the Soil and Water Research Infrastructure, Biology Centre of the Czech Academy of Sciences (České Budějovice, Czech Republic) using a MAT253 Plus isotope ratio mass spectrometer (IRMS) coupled to an elemental analyser (EA Isolink CNSOH) via a Conflo IV interface (all equipment from Thermo Scientific, Bremen, Germany). Samples were analysed together with the international reference material IAEA-600 and an in-house fish muscle working standard and data were reported in conventional delta notation, normalised to the international reference scale ($\delta^{13}C$-VPDB and $\delta^{15}N$-air, ‰). Repeated measurements of the reference standards indicated that the precision was < 0.2‰ and < 0.4‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Table 1. Sampling sites involved in the study, selected on the basis of the presence of crucian carp (*Carassius carassius*) and topmouth gudgeon (*Pseudorasbora parva*) in the syntopy, with additional presence of gibel carp (*C. gibelio*) at four sites. CC = crucian carp, TMG = topmouth gudgeon, GC = gibel carp. The site ID corresponds to Fig. 1.

Site ID	Sites	Macrophytes (%)	Latitude/ Longitude	Area (ha)	Sampling date	No. of individuals	Standard length (mm)	CPUE
1	Soudkuv lom	50	49.6280844°N, 15.3921969°E	0.04	14/6/2023	CC = 12 TMG = 14 GC = 15	CC = 105 ± 22 TMG = 40 ± 3 GC = 56 ± 25	CC = 0.16 TMG = 0.41 GC = 0.40
2	Lipi	100	48.9414672°N, 14.3579775°E	0.01	21/6/2023	CC = 15 TMG = 15 GC = 15	CC = 68 ± 10 TMG = 56 ± 7 GC = 48 ± 8	CC = 2.18 TMG = 0.93 GC = 1.59
3	U Spacku	100	48.9477278°N, 14.4882947°E	0.11	23/6/2023	CC = 15 TMG = 12 GC = 15	CC = 50 ± 6 TMG = 48 ± 5 GC = 56 ± 24	CC = 0.75 TMG = 1.45 GC = 0.95
4	Vrabce	30	48.9209244°N, 14.3631514°E	0.06	28/6/2023	CC = 15 TMG = 11 GC = 11	CC = 56 ± 9 TMG = 32 ± 3 GC = 109 ± 29	CC = 2.40 TMG = 64.5 GC = 0.10
5	Smilovice	10	49.8668344°N, 14.9854161°E	0.13	8/5/2023	CC = 15 TMG = 15	CC = 98 ± 10 TMG = 46 ± 3	CC = 0.30 TMG = 3.80
6	Valcha	55	49.7043211°N, 13.3039761°E	0.20	14/7/2023	CC = 6 TMG = 15	CC = 69 ± 9 TMG = 48 ± 6	CC = 0.07 TMG = 8.21

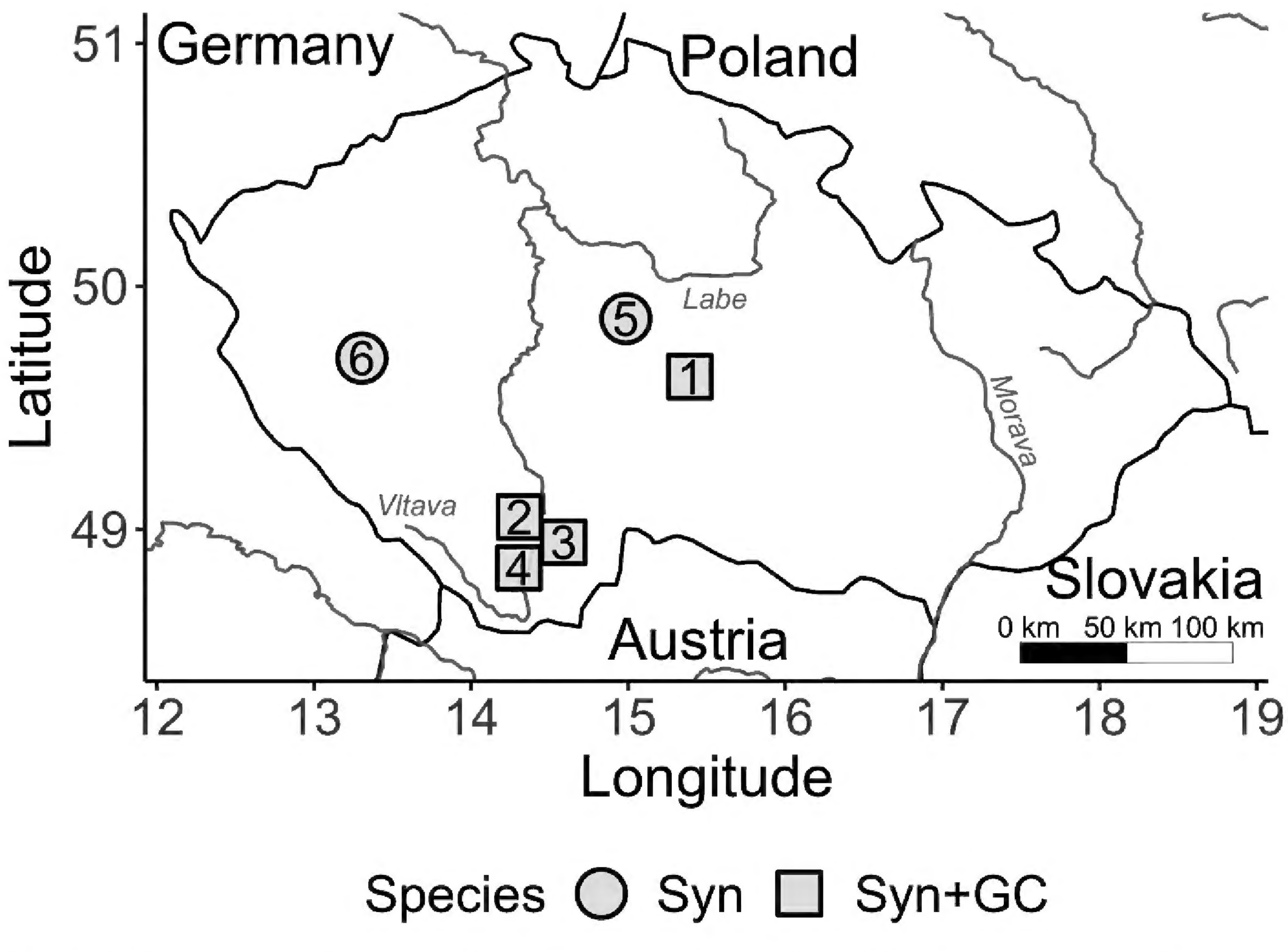

Figure 1. Map of sampling sites where specimens were collected for stable isotope analysis to estimate the trophic niche overlap of topmouth gudgeon (*Pseudorasbora parva*) and crucian carp (*Carassius carassius*). Species: Syn = topmouth gudgeon + crucian carp; syn+GC = topmouth gudgeon + crucian carp + gibel carp (*C. gibelio*). Site details are given in Table 1 with corresponding ID.

Statistical analyses

Mesocosm evidence

To assess the position of the isotopic niches of the tested species in syntopy and allopatry, standard ellipse areas (40% EA) were used, which are a bivariate measure of the distribution of individuals in isotopic space, where the ellipse includes the

mean 40% of the data as it represents the typical resource utilisation of the population (Jackson et al. 2011). In addition, the 95% ellipse area (95% EA) was plotted, which is a bivariate equivalent of the 95% confidence interval that includes 95% of the sample (Layman and Allgeier 2012).

A permutation analysis of variance with the adonis function (PERMANOVA, distance = Euclidean, nperm = 10,000) in the R package vegan was used to estimate the difference in syntopy between the isotopic niches of crucian carp and topmouth gudgeon (Oksanen et al. 2022). After confirming significance, analyses of variance (ANOVA) were performed to determine whether the significance of the isotopic niche was due to the $\delta^{13}C$ or $\delta^{15}N$ values (Balzani et al. 2020). In addition, the percentage of overlap between species in syntopy was calculated as two overlapping ellipse areas (Bayesian 40% or 95% EA) divided by the sum of the ellipse areas of both species ×100 according to the equation of Stasko, Johnston and Gunn (2015). The percentage of overlap was calculated for each posterior draw of the Bayesian estimates of overlap and ellipse areas to obtain the distribution with 1,000 iterative draws using the SIBER package. Finally, the directional pairwise probability of an individual of one species falling into the niche of another species was estimated at the 95% Bayesian ellipse area using the R package nicheROVER (Lysy et al. 2021) with a Monte-Carlo estimation (10,000 iterations).

Finally, following Layman et al. (2007), six isotopic niche metrics were calculated to assess the potential difference in the syntopy and single species treatment of the mesocosms. The $\delta^{15}N$ range (NR) and the $\delta^{13}C$ range (CR) as differences between the most enriched and the most depleted individual, the mean Euclidean distance of each individual from the centroid of the $\delta^{13}C$-$\delta^{15}N$ values (CD), the mean nearest neighbour distance in $\delta^{13}C$-$\delta^{15}N$ space (NND) and its standard deviation (SDNND).

Field evidence

Identical statistical and visual approaches as above were used to estimate the overlap between the isotopic niches of crucian carp and topmouth gudgeon at six syntopic sites, out of which the invasive gibel carp was also present at four of them. Statistical analyses and figures were generated using R version 4.2.2 (R Core Team 2022) with the R packages ggplot2, ggmap, SIBER and nicheROVER (Jackson et al. 2011; Kahle and Wickham 2013; Wickham 2016; Lysy et al. 2021).

Results

Mesocosm niche overlap

On average, the fish in the mesocosm increased in biomass during the experiments, with the highest increase observed in the crucian carp single species and syntopy treatments (248.4 ± 44.5% and 221.0 ± 55.2% of the initial biomass, respectively) and lower growth in the topmouth gudgeon single-species treatments (106.4 ± 25.4% and 107.8 ± 32.2%, respectively). Seven out of 16 treatments with topmouth gudgeon (both single species and syntopy) produced offspring of topmouth gudgeon, while crucian carp produced no offspring. Five topmouth gudgeon died during the experimental period, two in syntopy and three in the single species treatment.

Differences in species' isotopic niches

The syntopy treatment in the mesocosm showed that the mean isotopic values of topmouth gudgeon and crucian carp differed significantly (PERMANOVA: $F_{1,38}$ = 10.56, p < 0.001; Fig. 2A), with the topmouth gudgeon having higher $\delta^{15}N$ values ($F_{1,38}$ = 10.31, p = 0.002) and lower $\delta^{13}C$ values ($F_{1,38}$ = 8.56, p = 0.005) than the crucian carp. Isotopic niche size was significantly smaller for topmouth gudgeon in syntopy (95% EA = 12, 40% EA = 2) than in the single-species treatment (95% EA = 35, 40% EA = 6), while the isotopic niche size of crucian carp remained approximately the same (single 95% EA = 25, 40% EA = 4; syntopy 95% EA = 27, 40% EA = 5; Table 2, Fig. 3A), but shifted in orientation in $\delta^{13}C$-$\delta^{15}N$ space (Table 2, Fig. 2A). In the syntopy treatment, the $\delta^{13}C$ range of the crucian carp was smaller compared to the single species treatment, whereas this range was consistently low in topmouth gudgeon in both treatments. In contrast, the $\delta^{15}N$ range of topmouth gudgeon was reduced for fish growing in syntopy versus single species treatments and crucian carp exhibited a relatively larger $\delta^{15}N$ range in syntopy experiments. In comparison with the respective single species treatments, both species exhibited a lower mean distance to centroid, the topmouth gudgeon reduced the distance between nearest neighbours more than the crucian carp and crucian carp exhibited an increased standard deviation of nearest neighbour distance in syntopy treatments (Table 2).

Niche overlaps

The Bayesian overlap between the isotopic niches of crucian carp and topmouth gudgeon was 56% and 26% at 95% and 40% ellipse area, respectively (Fig. 2A). The individual probability (alpha = 95%) of entering the niche of the other species in the syntopy of crucian carp and topmouth gudgeon showed that topmouth gudgeon was more likely to enter the isotopic niche of crucian carp (90%) than vice versa (49%), which is in contrast with the field evidence below.

Field evidence

Differences in species' isotopic niches

The study sites differed significantly in their mean $\delta^{15}N$ values (PERMANOVA: $F_{5,210}$ = 232.4, p < 0.001; Fig. 2B), with the highest observed at the U Spacku and Lipi sites and the lowest at the Soudkuv lom and Vrabce sites. The highest $\delta^{13}C$ values were observed at the Soudkuv lom and Vrabce sites, while the lowest at the U Spacku site (Fig. 2B). Separate PERMANOVAs at each site showed that the differences between species were significant at all sites, except Valcha (Soudkuv lom: $F_{2,38}$ = 32.5, p < 0.001; Lipi: $F_{2,42}$ = 18.9, p < 0.001; U Spacku: $F_{2,34}$ = 12.6, p < 0.001; Vrabce: $F_{2,34}$ = 11.3, p < 0.001; Smilovice: $F_{1,28}$ = 25.4, p < 0.001; Valcha $F_{1,19}$ = 3.3, p = 0.066). The $\delta^{15}N$ values did not differ significantly between topmouth gudgeon and crucian carp at the sites where gibel carp was present as a third species, but did differ significantly at the Smilovice site, where only topmouth gudgeon and crucian carp were present (Table 3). The $\delta^{13}C$ values were higher for crucian carp than for topmouth gudgeon at the Lipi, Vrabce and Smilovice sites, while they did not differ at the Soudkuv lom and U Spacku sites (Table 3). Overall, invasive topmouth gudgeon was more likely to have similar isotope values to the native crucian carp than the invasive gibel carp (Table 3).

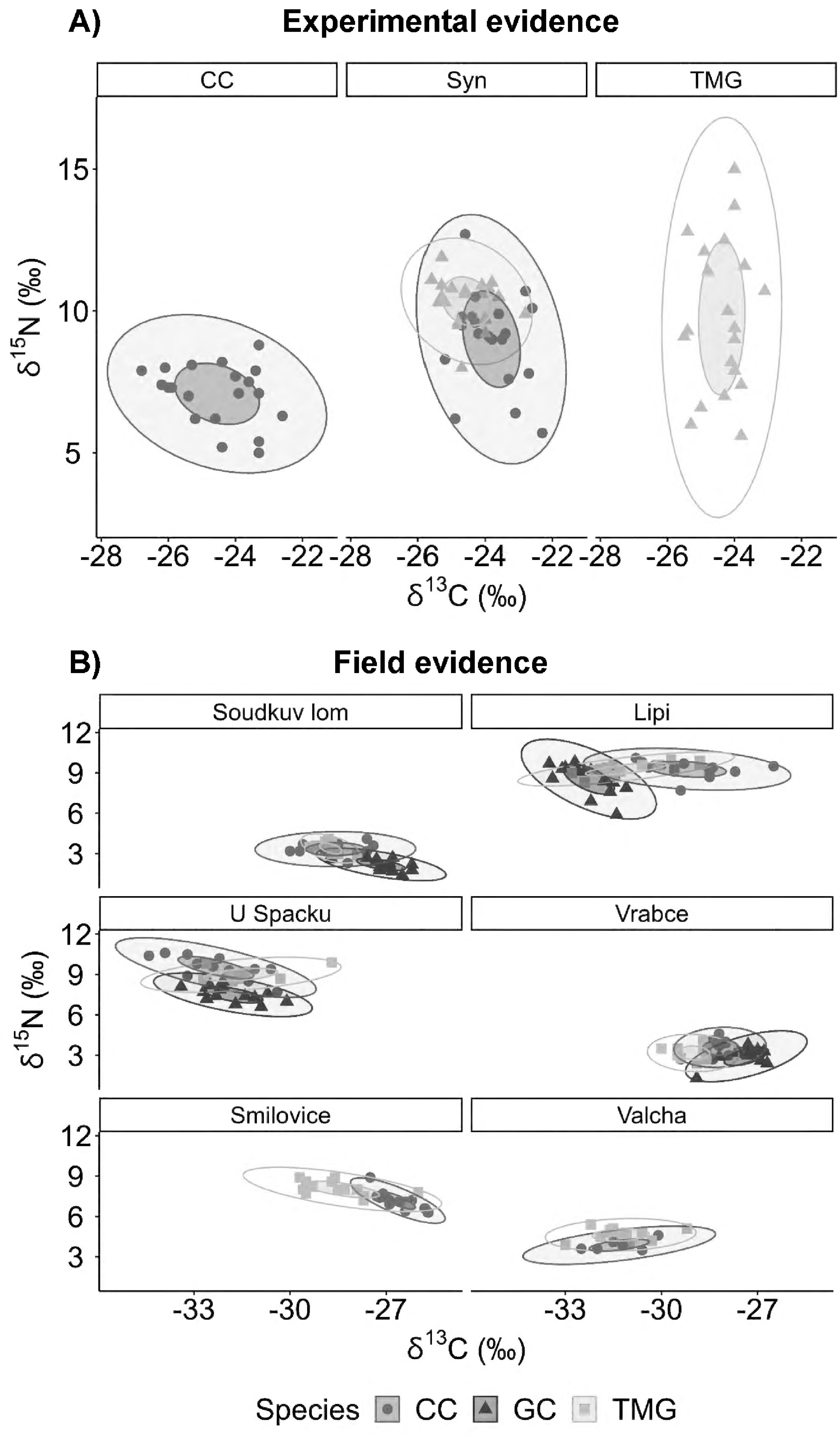

Figure 2. Mesocosm experiment (**A**) with treatments of crucian carp (CC, *Carassius carassius*), syntopy (Syn) and topmouth gudgeon (TMG, *Pseudorasbora parva*) and field evidence from six sites (**B**). Isotopic niches shown as 40% standard ellipse area for three species at each site (darker shading) and 95% ellipse area (lighter shading). Solid lines = ellipse areas, symbols = individual values. CC = Crucian carp (*Carassius carassius*), GC = Gibel carp (*C. gibelio*); TMG = Topmouth gudgeon (*Pseudorasbora parva*).

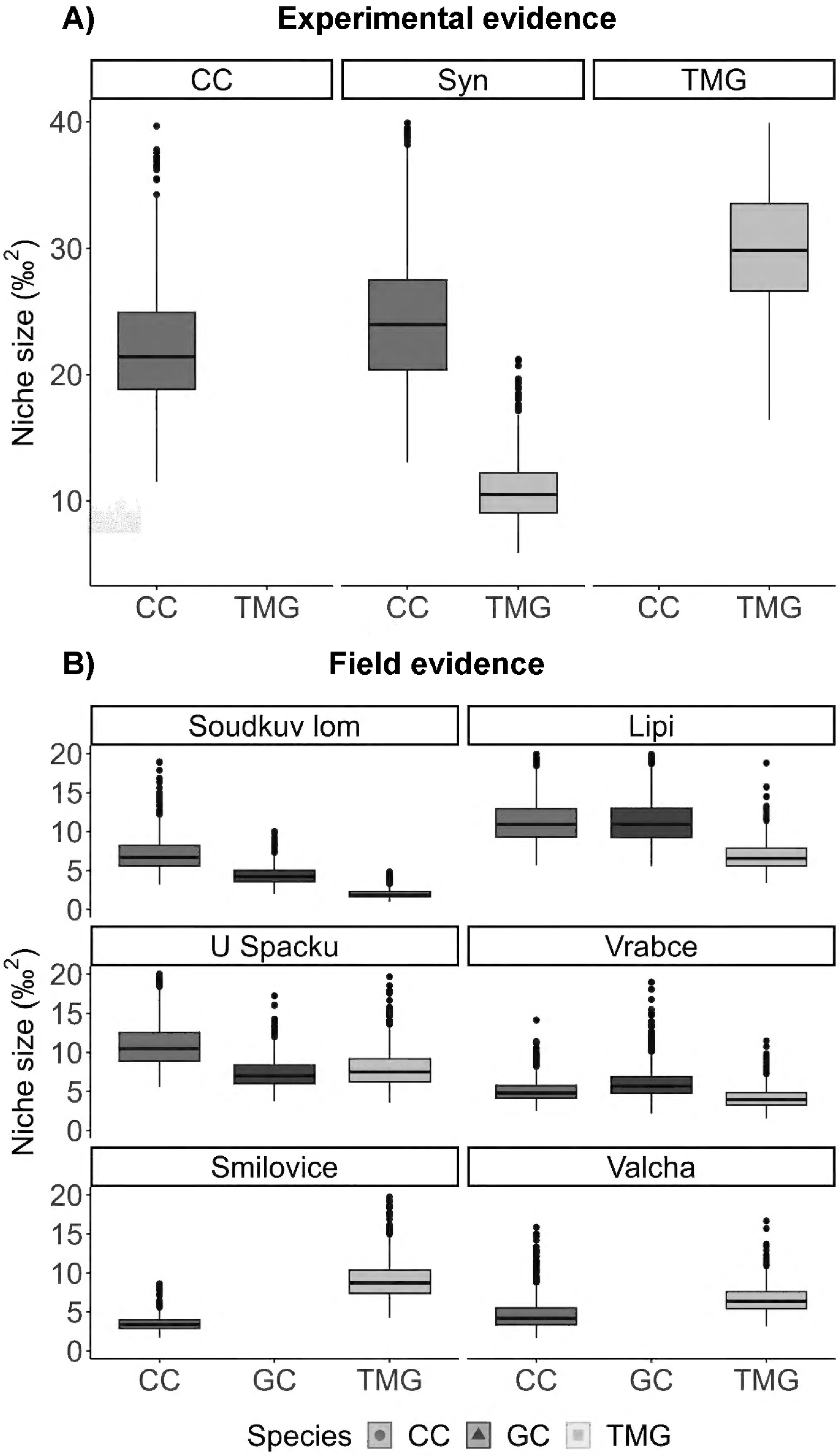

Figure 3. Posterior probability distributions of estimated Bayesian 95% ellipse area for mesocosm experiment and field dataset, modelled with nicheROVER package (Lysy et al. 2021). The isotopic niche of topmouth gudgeon (TMG, *Pseudorasbora parva*) was considerably reduced in syntopy (Syn), while the isotopic niche size of native crucian carp (CC, *Carassius carassius*) remained similar in the experimental setup (**A**). The topmouth gudgeon (TMG, *Pseudorasbora parva*) had a generally smaller isotopic niche size when compared to native crucian carp (CC, *Carassius carassius*) in the presence of gibel carp (GC, *C. gibelio*). In the field, its niche was substantially larger in syntopy only with native crucian carp (**B**). The boxplot boundaries represent upper and lower quartiles; the thick lines represent medians and the whiskers represent 1.5 times the interquartile range.

Table 2. Layman's metrics for isotopic niches of crucian carp (CC, *Carassius carassius*), topmouth gudgeon (TMG, *Pseudorasbora parva*) and co-existing gibel carp (GC, *C. gibelio*) in the mesocosm experiment and field evidence. CR = $\delta^{13}C$ range, NR = $\delta^{15}N$ range, CD = mean distance to centroid, NND = mean nearest neighbour distance, SDNND = standard deviation of nearest neighbour distance.

Mesocosm data						
Treatment	**Species**	**CR**	**NR**	**CD**	**NND**	**SDNND**
Single-species	CC	4.2	3.8	4.5	0.6	0.3
Syntopy	CC	2.9	7.0	1.5	0.7	0.6
Single-species	TMG	2.4	9.4	2.3	0.7	0.4
Syntopy	TMG	2.8	3.9	1.0	0.4	0.3
Field data						
Site	**Species**	**CR**	**NR**	**CD**	**NND**	**SDNND**
Soudkuv lom	CC	2.60	1.80	0.87	0.40	0.13
	TMG	1.50	1.20	0.45	0.17	0.15
	GC	2.50	1.50	0.69	0.28	0.11
Lipi	CC	4.30	2.40	1.11	0.43	0.39
	TMG	4.00	1.70	1.10	0.35	0.23
	GC	2.40	3.90	1.16	0.49	0.32
U Spacku	CC	4.00	2.90	1.15	0.55	0.28
	TMG	4.00	1.40	0.83	0.49	0.59
	GC	3.30	1.90	0.97	0.40	0.25
Vrabce	CC	2.20	1.90	0.65	0.32	0.25
	TMG	1.40	1.70	0.63	0.29	0.18
	GC	2.20	2.50	0.71	0.40	0.48
Smilovice	CC	1.80	2.60	0.64	0.30	0.29
	TMG	3.70	2.20	1.02	0.39	0.33
Valcha	CC	2.40	1.10	0.77	0.62	0.30
	TMG	3.80	1.60	0.72	0.48	0.40

Table 3. Results of Analysis of Variance (ANOVA) of $\delta^{15}N$ and $\delta^{13}C$ values at six sites and post-hoc Tukey tests. The Valcha site showed a non-significant permutation analysis of variance for $\delta^{15}N$ and $\delta^{13}C$; therefore, no ANOVAs were performed. CC = crucian carp (*Carassius carassius*), GC = gibel carp (*C. gibelio*); TMG = topmouth gudgeon (*Pseudorasbora parva*).

Site	ID		F	df	p	CC×TMG	CC×GC	TMG×GC
Soudkuv lom	1	$\delta^{15}N$	34.9	2	< 0.001	0.95	< 0.001	< 0.001
		$\delta^{13}C$	22.9	2	< 0.001	0.85	< 0.001	< 0.001
Lipi	2	$\delta^{15}N$	4.8	2	0.013	1.00	0.03	0.02
		$\delta^{13}C$	30.6	2	< 0.001	< 0.001	< 0.001	0.01
U Spacku	3	$\delta^{15}N$	38.7	2	< 0.001	0.1	< 0.001	< 0.001
		$\delta^{13}C$	2.0	2	0.152	–	–	–
Vrabce	4	$\delta^{15}N$	4.5	2	0.018	0.172	< 0.05	0.572
		$\delta^{13}C$	23.8	2	< 0.001	< 0.01	< 0.01	< 0.001
Smilovice	5	$\delta^{15}N$	13.8	1	< 0.001	–	–	–
		$\delta^{13}C$	28.5	1	< 0.001	–	–	–
Valcha	6	$\delta^{15}N$	–	–	–	–	–	–
		$\delta^{13}C$	–	–	–	–	–	–

Niche sizes and overlaps

The isotopic niche of crucian carp was generally larger than those of topmouth gudgeon and gibel carp at sites where all three species occurred, while the niche of topmouth gudgeon was larger than that of crucian carp at the Smilovice and Valcha sites, where only these two species occurred (Table 2; Figs 2B, 3B). The gibel carp generally had an intermediate isotope niche size compared to the other two species (Table 4; Figs 2B, 3B). The results of the Bayesian directional niche overlap analysis suggested that overlap varied between the sites studied and the highest probability at 95% α was that the topmouth gudgeon trophic niche occurred within the niche space of the crucian carp. This was followed by crucian carp overlapping the topmouth gudgeon isotopic space and then a relatively equal probability of invasive gibel carp occurring within native crucian carp and topmouth gudgeon isotopic space (mean TMG within CC = 74; CC within TMG = 47; GC within CC = 23; CC within GC = 19; GC within TMG = 20; TMG within GC = 27; Table 4). The sites where only topmouth gudgeon and crucian carp were present showed an inverse pattern, where crucian carp was more likely to be present in the topmouth gudgeon isotope space than vice versa (19 and 75, respectively; Table 4).

Table 4. Ellipse areas (EA) of the isotopic niche encompassing 40% or 95% of the data. The ellipse areas and their isotopic niche overlaps were calculated with the R package Siber using Bayesian posterior probability distributions (Jackson et al. 2011). The directional probability of individual overlap with a probability of 95% from the third column "Species" into the niche of other species (P of ind. overlap at 95 %) was calculated with the R package nicheROVER (Lysy et al. 2021). CC = crucian carp (*Carassius carassius*), GC = gibel carp (*C. gibelio*); TMG = topmouth gudgeon (*Pseudorasbora parva*).

Site	ID	Species	40% EA	40% EA overlap			95% EA	95% EA overlap			P of ind. overlap at 95 %		
				CC	TMG	GC		CC	TMG	GC	CC	GC	TMG
Soudkuv lom	1	CC	1.44	–	43.24	0	8.45	–	43.45	28.94	NA	22.53	45.68
		TMG	0.40	–	–	0	2.35	–	–	23.90	96.56	27.28	NA
		GC	0.86	–	–	–	5.09	–	–	–	27.61	NA	8.89
Lipi	2	CC	2.22	–	12.51	0	13.04	–	48.95	10.16	NA	3.8	41.59
		TMG	1.35	–	–	9.06	7.95	–	–	34.56	63.80	42.5	NA
		GC	2.26	–	–	–	13.27	–	–	–	6.73	NA	37.24
U Spacku	3	CC	2.21	–	36.49	0	12.96	–	52.56	20.04	NA	8.57	50.61
		TMG	1.61	–	–	0	9.43	–	–	31.48	73.1	20.79	NA
		GC	1.47	–	–	–	8.61	–	–	–	14.9	NA	20.58
Vrabce	4	CC	0.99	–	6.37	0	5.81	–	55.97	42.54	NA	40.25	49.95
		TMG	0.86	–	–	0	5.05	–	–	24.25	63.04	16.85	NA
		GC	1.27	–	–	–	7.46	–	–	–	41.73	NA	13.19
Smilovice	5	CC	0.69	–	0.08	–	4.09	–	42.01	–	NA	NA	71.82
		TMG	1.80	–	–	–	10.55	–	–	–	23.30	NA	NA
		GC	–	–	–	–	–	–	–	–	NA	NA	NA
Valcha	6	CC	1.20	–	4.94	–	7.06	–	54.43	–	NA	NA	61.16
		TMG	1.30	–	–	–	7.65	–	–	–	37.72	NA	NA
		GC	–	–	–	–	–	–	–	–	NA	NA	NA

Discussion

The data collected for this study indicate that the presence of the topmouth gudgeon leads to an overlap of isotopic niches with the native crucian carp, which confirms the first general hypothesis. Both in the field and in the mesocosm, there was evidence of strong overlap between the invasive topmouth gudgeon and the native crucian carp. A very important competitive interaction can occur between young-of-the-year crucian carp and the topmouth gudgeon, as the latter often reaches enormous densities and has a very strong influence on the availability of zooplankton (Musil et al. 2014; Kajgrová et al. 2022). The topmouth gudgeon feeds on pelagic zooplankton, on which the small crucian carp also depends (Holopainen et al. 1997; de Meo et al. 2022; Kajgrová et al. 2022). Therefore, the interaction of topmouth gudgeon with crucian carp can limit recruitment and lead to a decline of their population.

Trophic interrelationships amongst topmouth gudgeon, crucian carp and gibel carp

At three sites, Lipi, Vrabce and Smilovice, crucian carp was, on average, more enriched in ^{13}C than topmouth gudgeon. This suggests that crucian carp may utilise more aquatic littoral food sources than topmouth gudgeon at these sites, as the base of the aquatic littoral food web generally exhibits higher $\delta^{13}C$ values compared to the base of the pelagic food web (France 1995), partially confirming the second hypothesis. In addition, gibel carp exhibited higher $\delta^{13}C$ values compared to the crucian carp in Vrabce and Soudkuv lom, which is consistent with a previous study on the competition between these *Carassius* species, where the gibel carp was able to access food resources at lower trophic levels, with addition of plant sources compared to the crucian carp (Tapkir et al. 2023). Finally, apart from the U Spacku site, the carbon niche of the topmouth gudgeon and gibel carp were significantly different, with the latter utilising more littoral resources. The comparison between topmouth gudgeon and the two *Carassius* species showed an opposite trend at the Lipi site, which is an exception amongst the selected ponds in terms of macrophyte density, as the bottom is completely covered by *Ceratophyllum demersum* and the water surface by *Lemna minor*. The complexity of the environment and the proportions of littoral versus pelagic production may, therefore, influence the competition between the species, which provides a natural setting that may be useful to test future hypotheses.

The relative $\delta^{15}N$ values are an indicator of the trophic position of the species (Post 2002; Jackson and Britton 2013 Westrelin et al. 2023) and these generally did not differ between topmouth gudgeon and crucian carp during the field survey, with the exception of the Smilovice site, where topmouth gudgeon was positioned slightly higher. On the other hand, the gibel carp had significantly lower $\delta^{15}N$ values than the crucian carp at all sites and also significantly lower values than topmouth gudgeon everywhere, except the Vrabce site. These results, together with those of our previous study (Tapkir et al. 2023), show that the invasive gibel carp often occupies a lower trophic position in the food web, probably because it also uses plant food to some extent. In general, higher $\delta^{15}N$ values of aquatic organisms often indicate nitrogen pollution from increased land use (Vašek et al. 2023) and this effect is visible at the Lipi, U Spacku and Smilovice sites, which are located in

an agricultural landscape and the fish there had higher $\delta^{15}N$ values. In contrast, the Soudkuv lom, Vrabce and Valcha sites are located in a forest with little human impact and the fish there had significantly lower $\delta^{15}N$ values. In a future study, a detailed analysis of the food sources and their isotopic signals in different seasons could help to assess the niche partitioning between the study species.

The topmouth gudgeon had a very large isotopic niche in the single species treatment, whereas the syntopic treatment showed a flexible shift in its niche. This was only partly consistent with our third hypothesis and other studies (Britton et al. 2010; Jackson and Britton 2013; Balzani et al. 2020), as we expected that topmouth gudgeon will maintain a large isotopic niche under interspecific competition. Similarly, the field data also did not support the large isotopic niche of topmouth gudgeon in interspecific competition, as its isotopic niches were predominantly smaller than those of the other two species. The alternative trends observed in the current study may be due to the comparison of topmouth gudgeon with generalist, omnivorous *Carassius* species that exploit all available resources and, thus, also have a relatively large isotopic niche, whereas the study by Jackson and Britton (2013) compared topmouth gudgeon with species typical for more diverse fish communities. Furthermore, $\delta^{15}N$ values of topmouth gudgeon may have been influenced by spawning activity and digestion of eggs and larvae, which could have partially influenced mesocosm experiments and sites with high catch per unit of effort of topmouth gudgeon. Similarly, it was found that rudd (*Scardinius erythrophthalmus*) increased its trophic position through preying upon small topmouth gudgeon (Britton et al. 2010).

Regarding the overlap of isotopic niches, topmouth gudgeon overlapped more with the crucian carp than with the invasive gibel carp and the overlap with topmouth gudgeon was greatest in the presence of the second invasive species, the gibel carp, which confirms the fourth hypothesis. The crucian carp adapts its isotopic niche to the aquatic community (de Meo et al. 2022) and can co-exist with several species, including predators (Holopainen et al. 1997 Olsén and Bonow 2023). In response to predation, the crucian carp was demonstrated to increase its dependence on littoral food sources and included larger invertebrates in its diet (de Meo et al. 2022). However, competition with the topmouth gudgeon can be very intense and is new for this species. The typical sites are small waterbodies and alluvial pools, where a maximum of three species co-exist and there is no fish predator to thin out their populations, which would reduce inter- and intraspecific competition.

Complexity of topmouth gudgeon interactions with other species

In this study, we have only addressed part of the interactions between species, namely isotopic niche overlap, which was assessed via a stable isotope approach. However, an organism's niche consists of many different aspects of resource sharing in space and time (Schoener 1974; Jackson et al. 2011) and, thus, the insight into interspecific interactions is simplified by the approach used. Additionally, the negative impact of topmouth gudgeon on other fish species is specific due to the transmission of the rosette pathogen *Sphaerothecum destruens*, which has a negative impact on other native floodplain species, such as the sunbleak (*Leucaspius delineatus*) (Gozlan et al. 2005; Spikmans et al. 2020) and other fish species (Andreou and Gozlan 2016; Spikmans et al. 2020). The sunbleak is also classified as critically endangered in Czechia and the number of known populations is even lower than

that of the crucian carp (Chobot and Němec 2017). However, the authors are not aware of any evidence that the rosette pathogen also affects the crucian carp.

The topmouth gudgeon has also been found to harm other fish through parasitic behaviour (Oberle et al. 2019). We only observed missing scales in mesocosm experiments and all crucian carp survived the syntopy mesocosm experiment, but this may be due to the relatively low density of topmouth gudgeon, whereas the study by Oberle et al. (2019) was conducted at a high fish density relevant for pond aquaculture (Musil et al. 2014). Such densities can also be relevant in some waters not used for aquaculture and the Vrabce, Smilovice and Valcha sites were numerically dominated by topmouth gudgeon.

Interactions with multiple invasive species

Although we tried to study the competition between the two species, topmouth gudgeon and the crucian carp, it turned out that the fish communities very often also contained gibel carp and we were not able to obtain samples from the field where only the two former species co-occur. Gibel carp have very similar habitat requirements to crucian carp, with adaptation to anoxia conditions (Blažka 1958; Lushchak et al. 2001), morphological change of the body in response to predation (Tarkan et al. 2023) and they are very widespread in Czechia (Lusk et al. 2010; Lusková et al. 2010). Therefore, future studies of invasive fish should analyse habitat requirements and species limits during extreme events (e.g. anoxia in winter, drought in summer), which may modulate competitive outcomes between crucian carp and other fish species.

Conservation implications

Crucian carp populations have declined considerably in Europe (Kottelat and Freyhof 2007; Jeffries et al. 2016) and in countries with intensive aquaculture, such as in Czechia and their decline is largely associated with the spread of invasive fish species (Lusk et al. 2010; Tapkir et al. 2022). While the main focus has been on the gibel carp, which is a strong competitor for shared food resources (Tapkir et al. 2022, 2023), other fish species whose habitat requirements overlap with those of the crucian carp may also have contributed to the local extirpations. The topmouth gudgeon can reach astonishing densities, especially in productive eutrophic waters and consume a large portion, if not all small zooplankton (Musil et al. 2014; Kajgrová et al. 2022). It is possible that this ability and the dominance of topmouth gudgeon in the ecosystem may also limit the recruitment success of crucian carp.

The six sites selected for this study were part of the verification of the citizen-science project “Save the Crucian Carp” (Šmejkal et al. 2021), in which citizens provided information on the presence of the native crucian carp and the invasive gibel carp. In monitoring more than 200 sites for that study, the team of authors observed that many sites also contained topmouth gudgeon. Compared to the occurrence of crucian carp and gibel carp in syntopy, co-existence with topmouth gudgeon appears to be less common. Most sites appear to be relatively recently invaded by topmouth gudgeon, which may be explained by strong interactions between the species that prevent co-existence. In very dense populations of topmouth gudgeon, such as in Smilovice, there seem to be no small individuals in crucian carp populations. This could indicate that topmouth gudgeon can restrict reproduction to such an extent that crucian carp populations die out, but more research needs to be done in this direction.

The decline of the crucian carp has led to conservation actions to protect this species in England (Copp and Sayer 2010; Sayer et al. 2020), Belgium (Auwerx et al. 2021) and Czechia (Šmejkal et al. 2024). This study, as well as other evidence of interactions with other invasive fish, such as goldfish *Carassius auratus* (Smartt 2007 Tarkan et al. 2009, 2010; Copp et al. 2010), gibel carp (Auwerx et al. 2021; Tapkir et al. 2022, 2023) or Chinese sleeper *Perccottus glenii* (Reshetnikov 2003 Šmejkal et al. 2023b), indicate that these invasive fish species should be excluded from the community in drainable waterbodies and precautions taken against their invasion (e.g. minimising stocking practices, informing the public about their negative impacts) in order to maintain viable populations in protected waters.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

The field sampling methods and experimental protocols used in this study were performed by the guidelines and permission from the Ministry of Environment of Czechia (ZN/MZP/2022/630/1428).

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Author contributions

Marek Šmejkal: Conceptualisation, Investigation, Data curation, Formal analysis, Writing – Original draft, Writing – Review & Editing, Visualisation. Kiran Thomas: Data curation, Investigation, Writing – Review & Editing. Zuzana Šmejkalová: Data curation, Writing – Review & Editing. Yevdokiia Stepanyshyna: Investigation, Writing – Review & Editing. Sandip Tapkir: Data curation, Writing – Review & Editing. Travis Meador: Investigation, Formal analysis, Writing – Review & Editing. Mojmír Vašek: Investigation, Formal analysis, Writing – Review & Editing.

Author ORCIDs

Marek Šmejkal https://orcid.org/0000-0002-7887-6411
Kiran Thomas https://orcid.org/0000-0002-4318-1732
Daniel Bartoň https://orcid.org/0000-0001-8042-4564
Sandip Tapkir https://orcid.org/0000-0003-3456-8406

Travis Meador https://orcid.org/0000-0001-8255-5883
Mojmír Vašek https://orcid.org/0000-0001-6386-4015

Data availability

All of the data that support the findings of this study are available in the main text.

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